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The Watermark Disease

of the

Cricket-bat Willow

(Salix caerulea)

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BY

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ERRATA

PAGE 12, Fig. 9, read 'rays' for 'saps'.

PAGE 14, Fig. 11, delete all after 'twig'. Add 'A typical distribution of bacteria. They occupy only the minority of the vessels'.

PAGE 23, line 29, read 'water' for 'wate'.

PAGE 30, in reference Elwes and Henry, read 'Vol. vii' for 'Vol. iii'.

Oxford Forestry Memoir, No. 3.

) THE

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My thanks are due to Mr. A. A. Kempin for taking the microphotographs.

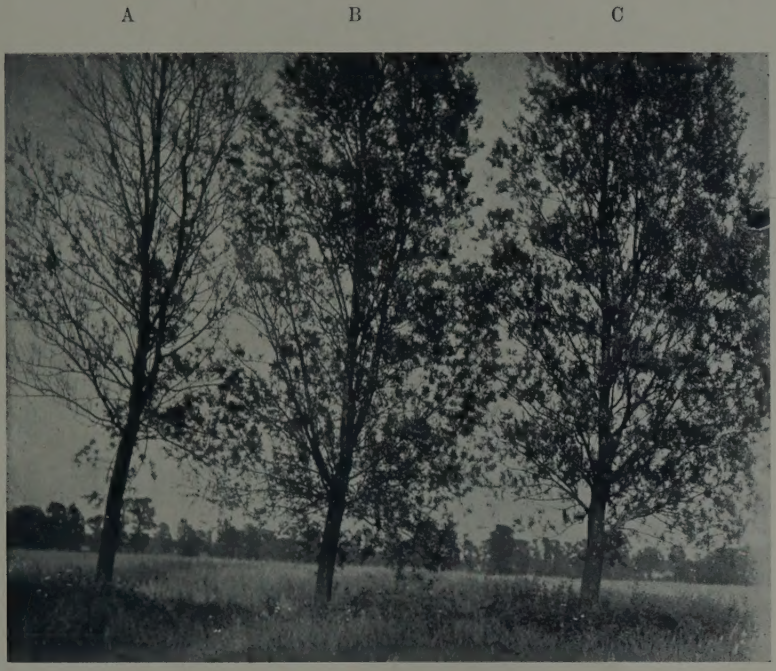

FIG. 1

Trees newly dying back. Only A and B are affected. It will be seen that B is only dying where it is in contact with A, suggesting that infection has spread from A to B. Photographed July 1923.

FIG. 2

Dead trees with bark fallen off the trunk. Photographed November 1922.

INTRODUCTION

The disease of *Salix caerulea*, a description of which is given in the following pages, is the cause of serious loss to willow growers engaged in the economic exploitation of this tree. In 1921, the prevalence of the disease in the valley of the River Chelmer was brought to the notice of Mr. W. E. Hiley, Research Officer at the School of Forestry, Oxford, and at his suggestion I undertook the investigation of it. The general symptoms of the disease are a dying back of the crown, which begins during the late spring. Even in the first year of attack a tree is so affected as to lose much of its value as timber; and such is the progress of the disease that, within one or two years, trees become a total loss. The disease appears to be confined to the genus *Salix*; and so far as the present investigation goes to *S. caerulea* and *S. alba*. *S. fragilis* also occurs in affected districts, but has been found so far to be free from disease.

DESCRIPTION OF DISEASE

In describing the disease three stages are recognized, the first stage, the subsequent stages, and the death of the trees. The first stage ends with the withering of the leaves on the dying-back branches, and the subsequent stages deal with those phenomena that occur up till the death of the tree.

(*a*) EXTERNAL SYMPTOMS

First Stage. In any one year, the disease may be recognized in May soon after growth has commenced. The first sign of disease, which usually develops before the new shoots are more than half grown, consists in the withering and death of the leaves and of the tips of the new shoots, on one or more branches of the dying-back crown. The leaves become brown and may remain on the tree for some considerable time. There is no regular order in the death of the branches, but I have noticed very frequently that the first branch to show signs of infection is one that is densely shaded. On the other hand, the disease may declare itself in branches suffering no disadvantage of position. The leaves on any one twig die as a whole, but the dying back on each branch is usually progressive, so that the leaves wither on one part after another. The whole crown is never affected, usually one, two, three, or four branches die back; but as indicated in Fig. 1, most branches of a crown may occasionally show signs of infection. When a number of branches are affected they may die back at one time or in succession. After July no

further branches die back, either on trees already diseased or those hitherto immune.

Concurrently with the withering of the leaves two things may happen: (*a*) adventitious shoots may develop both on the dying branches and on other parts of the tree; (*b*) exudations of bacteria may occur. The development of adventitious shoots takes place in a perfectly normal manner and need not be further discussed. Those shoots on the dying branches soon cease to grow and die back with the branch. The exudations only occur on dying branches and have their origin in the wood. They flow from the twigs and never from the larger branches. They always flow through wounds made by some external agent, and consist of a thin colourless transparent sticky liquid which covers the surface of the branch with a shiny varnish-like layer. It can easily be shown that they have their source in masses of bacteria inhabiting the vessels, and they occur in hot dry weather no less than in rainy periods. Unless washed away by rain, or used as a habitat by various fungi and other organisms, they gradually set hard and take on a yellow or brown colour. In a fresh state they are always thin and running and never take the form of stiff viscid masses or cirri, as sometimes do exudations accompanying bacterial diseases.[1]

The wounds through which the exudations flow are usually made by the imagos of small hymenopterous insects in boring their way out after pupation. These insects favour the one- and two-year-old branches and pupate in the pith or between the pith and the wood. The imagos bore out directly through the wood, or, as often occurs in one-year-old twigs, through the medullary parenchyma leading to one of the collateral accessory buds to be found on willows. The exudations appear to take place only through wounds made in the current season. They do not occur through old although open wounds, no doubt because in these the vessels from which the exudation is discharged are already blocked up. In their fresh state insects of various kinds can be found on them, the more common being green and black aphides.

During this stage of the disease, no fungal infection necessarily occurs; indeed, immediately upon the withering of the leaves, it is impossible to detect the presence of any fungus that might cause the dying back to begin.

Subsequent Stages. Subsequently to the withering of the leaves the affected branches slowly die back, beginning with the twigs. The dying parts become infected with a fungus, *Cytospora chrysosperma*, which produces its *pycnidia* in great numbers as the bark dies. The extent of the dying back is determined by the formation of a cork layer which develops during the autumn. Early in this season some branches on trees already diseased exhibit the phenomenon of a premature leaf-fall. The leaves die gradually, beginning with the lower ones on each twig, but the branches show no further sign of disease during the same year, and after the leaves have fallen appear to be quite healthy.

This phenomenon is the last external symptom of disease to be observed during any one year.

[1] e. g. Bean blight due to *Bacterium malvacearum* and Mulberry blight due to *Bacterium mori* —refer E. F. Smith, *Bacterial Diseases of Plants*, pp. 282 and 342.

Fig. 3
Watermarked branches off a freshly dying tree—collected and photographed in July 1922. Full size.

Fig. 4
A trunk watermarked on one side only, and with a very badly watermarked branch on that side. Photographed August 1922 within twenty-four hours after felling—the colour change in the watermark and its exudation is complete and is responsible for the darkly stained wood.

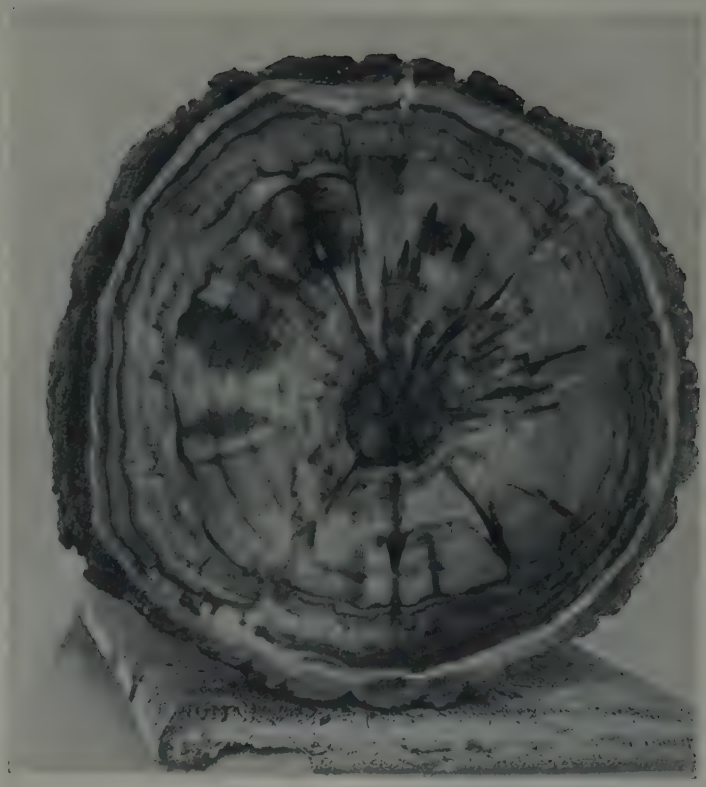

Fig. 5
Section of the trunk of a tree in the last stages of disease. The whole centre of the wood has become waterlogged.
Photographed November 1922.

The disease continues to spread within affected trees, and during succeeding years the phenomena that are described above are repeated. The rate of spread becomes slower as time goes on and sometimes badly affected trees cease to die back. However, in all cases they have become so injured as to make all recovery of normal growth impossible.

Death. This may occur within a year or two, or may be delayed for a number of years. From an economic point of view it is unimportant, as trees once attacked are of little or no value. After death has taken place fungi are found to have spread over the whole tree; and the bark of the trunk and larger branches becomes loose and falls off (Fig. 2), owing to the growth of mycelium between the bark and the wood.

(*b*) THE WATERMARK

The feature by which this disease can be distinguished from all others causing a die-back of willows, is to be seen on cutting through one of the dying branches. When this is done a dark mark, which is known locally in Essex as the 'watermark', is observed in the wood (Fig. 3). It appears in the still living parts of the tree, and after death has occurred gradually disappears, leaving only a faint grey stain. The watermark, the extent of which can be taken as indicating the progress of the disease throughout the plant, is due to bacteria which inhabit the vessels and are its cause.

First Stage. In a tree freshly dying back, the watermark can be traced throughout the dying branches and from them into the main stem of the tree, in which it extends upwards to some extent, but downwards to a much greater extent, usually, but not always, entering the root. It occupies the one-year-old annual ring and to a slight extent the autumn wood of the older adjoining ring. In the dying branches the current year's ring is also occupied, although perhaps only around the base of the new shoots; and in some cases the watermark may occupy the entire wood. The whole annual ring is usually watermarked in the smaller branches, but in the larger parts one side only may be affected (Fig. 4); and when this is so it can be seen that as the watermark spreads upwards or downwards it twists, in an anti-clockwise direction (looking at the roots) as shown in Fig. 6. This phenomenon can also be traced in Fig. 7, which shows the normal development of the watermark in the stem of freshly dying trees.

FIG. 6.—Diagram showing how the watermark twists in spreading through the main stem. The black branches are watermarked and the dotted lines indicate that they arise on the far side of the trunk. Data collected May 1923 from a newly dying tree. The scale of the diameter is three times that of the height of the tree.

The death of the leaves takes place only when the watermark has spread

from the one-year-old wood into that of the current year at the base of the new shoots. The upper part of these is rarely marked to any extent, and so far no bacteria have been discovered in the vascular system of the leaves or of the finer rootlets.

As the watermark extends through the main stem it ascends the hitherto

FIG. 7.—Diagrams showing the development of the watermark in the main stem of newly dying trees. In both cases the watermark is confined to the one-year-old ring and in each case shows the twist represented in Fig. 6. Only one branch had died back on each tree. Scale $1\frac{1}{2}'' = 1'\ 0''$. The figures give the height above the stool.

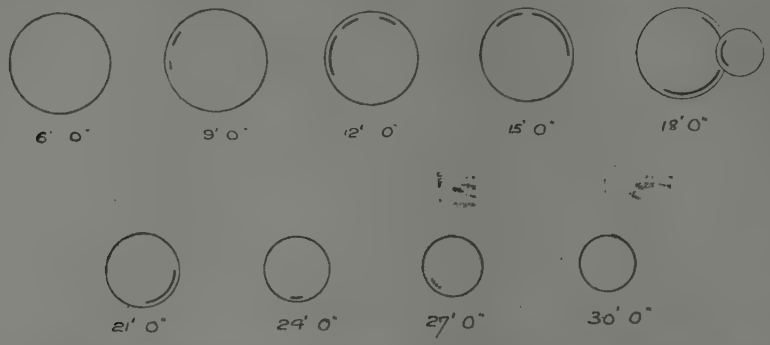

FIG. 7 A.—The affected branch entered at 26′ 0″. At 18′ 0″ the watermark is ascending a side branch. Age of tree 16–17 years from planting of set. Height approx. 50′.

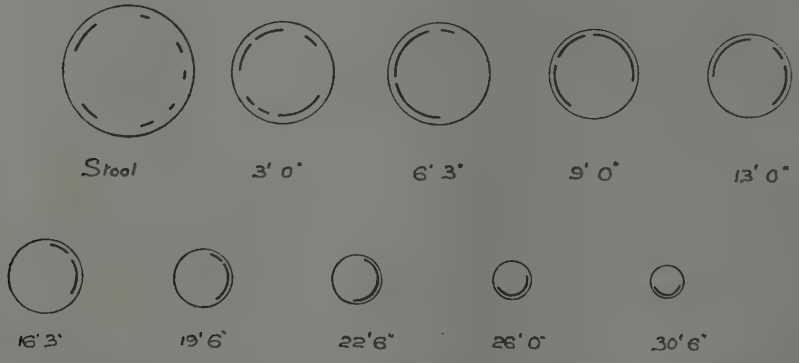

FIG. 7 B.—The affected branch entered at 20′ 6″ and was only a small sucker 2′ 6″ long put up during the previous year from the main stem. Age of tree 9 years from planting of set. Height approx. 45′.

unaffected branches, going first up the upper or lower side according to the direction in which it is spreading. During this stage only the base of these branches is affected.

The development of the watermark begins in the branches that die back first, and has chiefly taken place by the time the leaves wither; during the remainder of the season there is only a slow growth of bacteria. The rate of spread is most rapid in a longitudinal direction, towards the roots. The watermark spreads much more slowly into the crown and continues to develop

therein throughout the summer. The figures given in Table I show the extent to which it had spread in the main stems of trees selected because only one branch on each was dying back, and it was therefore easy to tell how far the disease had spread. It will be seen that there is no constant proportion between the rate of spread in an upward and a downward direction.

TABLE I

1	2	3	4	5	6	
Tree Number.	*Height in feet.*	*Diameter at Breast Height.*	*Height at which watermarked Branch joined Trunk.*	*Length of Ascent from 4.*	*Length of Descent from 4.*	*Remarks.*
	ft.	in.	ft. in.	ft. in.	ft. in.	
A	50	$7\frac{3}{4}$	26 0	2 6	18 6	Watermark confined to crown and main stem.
B	45	$7\frac{3}{4}$	20 6	14 6	20 6	Watermark entered roots in all cases. Col. 6 gives descent to stool only.
C	—	$7\frac{1}{4}$	14 0	3	14 0	
D	—	$6\frac{1}{4}$	13 0	4 0	13 0	
E	—	$9\frac{3}{4}$	12 0	2 0	12 0	

The rate of spread in a directly transverse direction is exceedingly slow, whether it takes place radially or tangentially. An example may be taken from tree Ref. A (Table I and Fig. 7 A). In this tree, at a height of 24 feet, two feet below the junction of the dying branch with the main stem, the watermark occupied one point only of the one-year-old annual ring. It was 1 inch in width and $\frac{3}{8}$ inch in thickness. From this point it had spread 16 feet 6 inches downwards, keeping to the same annual ring. These figures show that the rate of spread in directly transverse directions is negligible compared with that in a longitudinal direction. The watermark admittedly extends considerably, round the annual ring, as it descends the tree, but this is due to the longitudinal rather than the directly transverse spread of the disease. It is to be noted that in both Fig. 7 A and B, the watermark in no case entered the current year's wood, although it ran parallel to it for twenty or thirty feet. This is always so at this stage of the disease, and suggests that the bacteria either find it impossible to spread from the one-year-old annual ring directly through to the current year's ring, or that they do spread so very slowly that it is only noticed at a later period.

The development of the watermark within the root is similar to that within the crown and therefore need not be discussed. At this stage of the disease its colour is that of 'sap-logged' wood, with perhaps a slight tendency to turn a dark brown in the centre of the diseased areas. When a watermarked branch is cut through, an exudation, similar to that which occurs naturally from dying branches, comes from all parts with a sap-logged colour. In proportion to the size of the part cut through, this exudation is usually copious. Similarly to that which occurs naturally it turns brown, and so stains all the unmarked wood it runs over (Fig. 4). The watermark itself also changes colour on exposure to air, eventually becoming a very deep brown or black.

Subsequent Stages. These will be dealt with in relation to (*a*) the still living parts of the crown, (*b*) the dying branches, (*c*) the main stem.

(*a*) In the still living branches the development of watermark is very slow and takes place from the base upwards. It is confined to the one-year-old annual ring, and only those branches which arise from a watermarked side of the main stem are affected. During the first year none of these branches die back, but the phenomenon of premature leaf-fall is due to this slow spreading of the disease. In the larger parts of branches affected by this the watermark is well developed, but little or no trace of it is to be found in the twigs, and the current year's wood of all parts is free from bacteria. Many diseased trees do not exhibit this phenomenon, which appears to be confined to those in which the watermark spreads most rapidly.

As the crown dies back during succeeding seasons, the disease shows a similar development to that described in the first stage, except that from the first withering of the leaves more than one annual ring is watermarked. The dying back is sporadic, showing that the disease spreads unequally in different branches. Only when the final death of the tree occurs do the remaining live branches die back during one season.

(*b*) In the dying branches the development of the watermark is shown chiefly in the current year's wood, which now becomes decidedly stained, although not to such an extent as the one-year-old wood.

(*c*) The diagrams in Fig. 8 represent the probable manner in which the watermark, which is represented by the dotted line, spreads during the first twelve months after dying back begins. The watermark does not normally develop in the current year's wood of the main stem, which is thus left open to carry sap to the still living branches. In very badly attacked trees the outermost annual ring may become affected, and then the crown dies back in so far as this has occurred.

In a tree which has been diseased for two or more seasons, it is common to find that the central tissues of the larger branches and to some extent of the main stem have become deeply stained. This is a secondary effect associated with organisms following on after the pathogen and with changes induced in the wood. On very wet soils these organisms may develop to such an extent as to cause the whole centre of the main stem to be waterlogged (Fig. 5). In this case the wood gives out a faecal odour not associated with the pathogen.

After the first stage is completed a change in the colour of diseasded wo can be noticed. This is exactly similar to that already described as taking place on the watermark being exposed to the air during the earlier stages, but it takes much longer to develop, only being complete by the Autumn or Winter, and it is not seen in those parts in which the disease is still spreading.

Death. When this occurs, the watermark is always developed in the current year's wood of the main stem. The time required to kill a tree depends chiefly on how quickly this takes place, and inevitably varies according to the tree and the locality in which it grows.

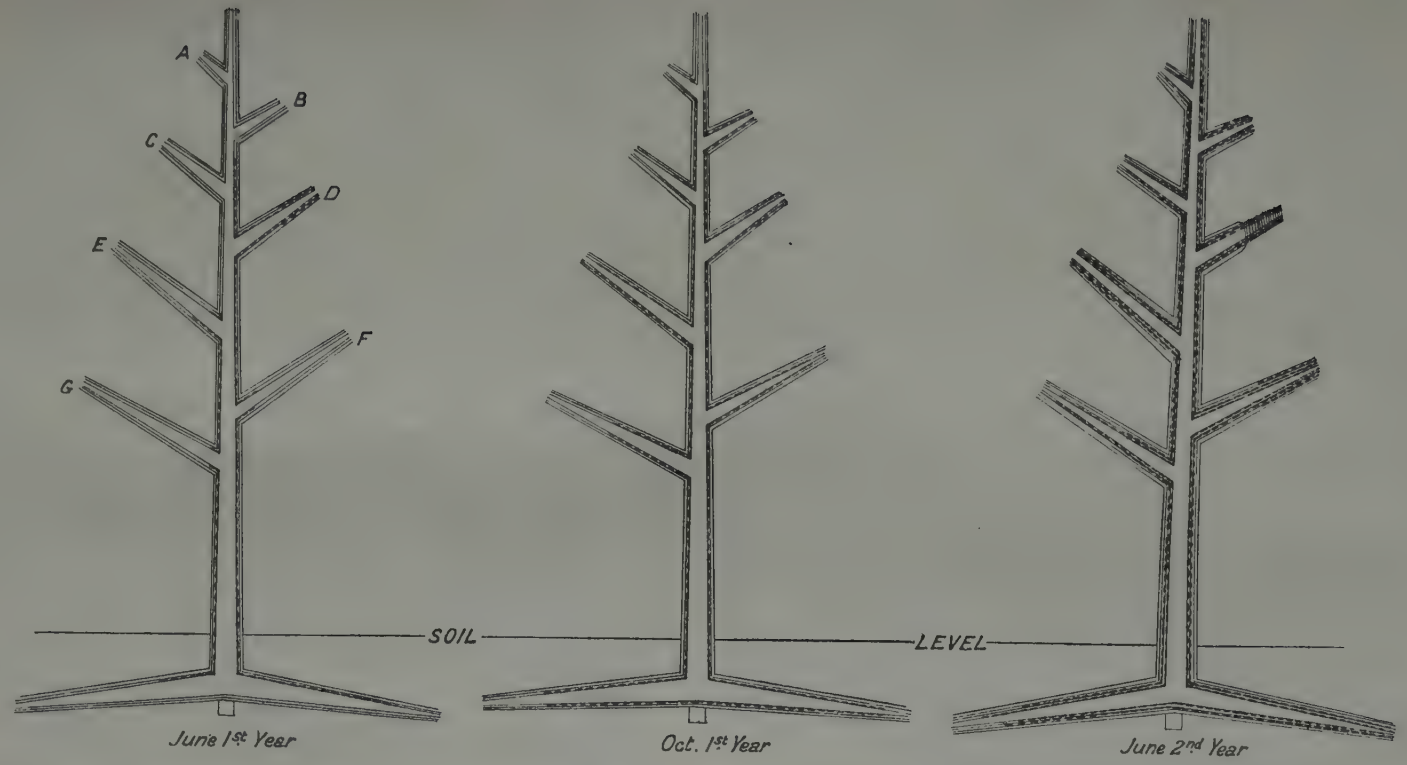

FIG. 8.—Diagrammatic representation of the spread of the watermark. The disease entered through the branch *D* which alone died back during the first year. By October the watermark has spread farther up the branches *B*, *E*, and *F* than any others: one or more of these may show the phenomenon of premature leaf-fall. By June of the second year *B*, *E*, and *F* have died back and the disease continues to spread in the remainder of the crown. This representation is partly theoretical.

(*c*) HISTOLOGICAL EFFECTS

The histological effects of the disease will be dealt with in the following order:

(*a*) The mechanical blocking of the vessels with bacteria;

(*b*) The effects upon the parenchyma in the wood;

(*c*) The production of pigment within watermarked areas;

(*d*) The effect upon the bark of dying branches.

(*a*) The first effect of the disease is that some of the vessels become blocked with bacteria (Figs. 9 and 10), and upon this the development of the watermark depends. Only the minority of the vessels are so affected (Fig. 11).

In the twigs the bacteria first block the vessels surrounding the pith (Fig. 9), the outermost cells of which normally contain large stores of starch; but in the larger branches no preference is shown for any part of the annual ring, and it is to be noted that the distribution of the starch is much more even.

(*b*) The parenchymatous tissues of the wood show the effect of the disease in three ways: (1) by the production of tyloses, (2) by the failure to carry normal stores of starch, (3) by the death of the cells. (1) Tyloses are produced both within and beyond the watermarked areas. This is an abnormal occurrence not shown by healthy wood. They are to be found when the leaves first wither, but the full extent of their development is only to be seen later in the year, when they may form a compact pseudo-parenchyma (Figs. 12 and 13). Their development does not depend upon the presence of bacteria within the vessel into which they grow. They are formed beyond the watermarked areas and also in these, where they occur in both blocked and unblocked vessels.

(2) At first a little starch may be found in the wood of dying branches; but eventually none is present either within or without the watermarked parts of the wood. Throughout the whole tree wherever watermark appears there is no starch present.

(3) The parenchyma cells within watermarked areas gradually die. The first to be affected are the medullary ray cells adjoining the vessels blocked with bacteria: they are easily detected by their brown colour. The bacteria do not appear to enter these cells, the death of which must therefore be brought about indirectly.

(*c*) The change in colour of the watermarked areas and of the exudation that flows from them is due to a brown pigment. The following things are known regarding this: (1) it is not produced by the pathogen; (2) a substance from which it is derived is present in the earlier stages of the watermark, for the pigment forms rapidly on exposure to air of watermarked wood as of the exudation from it; (3) it forms slowly within the wood, without direct exposure to the air, and thus produces the colour change therein; (4) it is not tannin, although this substance often appears as the disease progresses; (5) it is not formed in the non-watermarked parts nor in the wood of healthy trees; (6) a pigment exactly similar in colour forms slowly in a boiled extract of healthy wood (which is free from tannin); (7) the medullary ray cells in healthy wood turn brown upon being killed.

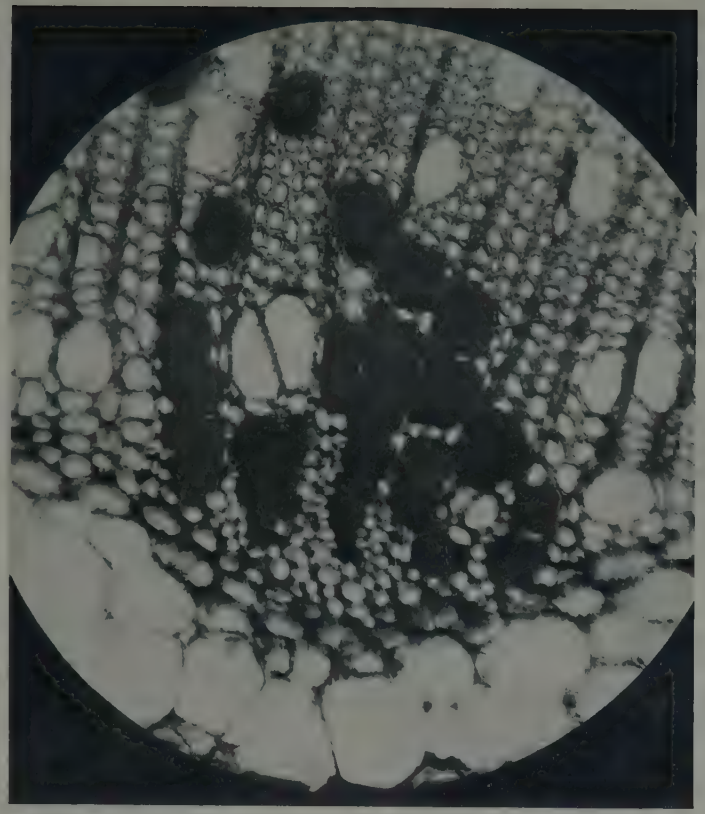

FIG. 9

A group of vessels near the pith of a two-year-old twig blocked up with bacteria. Note the absence of starch in the medullary saps and outer cells of the pith. Stained iodine and mounted in glycerine.

FIG. 10

A column of bacteria blocking a vessel. The column is broken in two places.

The above points indicate that the pigment is derived from some substance liberated from the parenchyma and that an oxidation process probably takes part in the formation of the pigment.[1]

(*d*) Little or no starch is found in the bark of freshly dying branches. Eventually there is none as far as the cork layer which cuts such branches off from the rest of the tree. The cork layer itself, finally, has to be noticed. Its formation does not depend on the presence of fungi that afterwards infect dying branches, but is a normal result of the progress of the pathogen throughout the plant.

(*d*) INFECTION

Infection takes place in the branches that first die back, and spreads from crown to crown. The evidence of this is:

(1) The occurrence of trees in which the watermark is confined to the crown and stem (as in Fig. 7 A).

(2) The dying back, at first, of only one branch situated in the middle of the crown. This often occurs; the bacteria would hardly spread from the root to such a branch without first entering those lower down the stem.

(3) The watermark first ascends the upper side of all unaffected branches below, and the lower side of all similar branches above those dying back. This is especially clear when only one branch dies and indicates that the pathogen spreads into the trunk from that branch. If it entered through the root, the lower side of all branches would become watermarked first.

(4) It has never been found that the root but not the crown is watermarked.

(5) Cases such as that photographed in Fig. 1 suggest that infection can spread from a badly diseased tree to its neighbour.

(6) The disease has been successfully reproduced by artificial infection in the crown of a pollard willow of some size (see later).

I believe the pathogen enters the host only by wounds which penetrate through the bark to the wood. No evidence has been obtained which shows that it enters through stomata, or hydathodes, or through wounds which affect the parenchyma only. Very probably the insects responsible for the holes through which the exudations flow also provide the means of ingress for the pathogen. In a disease-infected district bacteria-contaminated wounds occur very frequently. Even before pupation is completed insect wounds may be contaminated, and cases have been found in which the only possible means of ingress for bacteria was the wound made by the ovipositor of the insect. It has not been possible to show that the pathogen entered through such a wound, but it can hardly be doubted that disease does enter in this way, especially considering the ample sources of infection provided by the exudations.

[1] In connexion with this W. R. G. Atkins, in *Some Recent Researches in Plant Physiology* (pp. 188, 190), refers to the formation of a brown pigment in sap extracted from wood by means of crushing. When the extraction was made by centrifuging no such colour change occurred, indicating that the pigment had its origin in some substance liberated from the parenchyma when the wood was crushed. In these experiments an oxidase was present, but no such enzyme appears to be present in the exudation from the watermark.

The manner in which infection is carried is still doubtful. It is almost certainly spread by wind and rain or by direct droppings from the exudations. Insects which suck these also provide suitable carriers and possibly some insects may definitely emerge from a diseased stem and so be covered with infection. The green and black aphides which are quite common on exudations do not appear to spread the disease. This is possibly because the wounds they make affect the parenchyma and not the vascular system.

The dying back begins between May and July, and I believe that infection similarly takes place during a definite season which somewhat antedates this. This is not because infection cannot take place later. It has been shown experimentally that a tree can be infected at the end of July, with the result that it died back in the following September. In the field no fresh trees die back at such a time, and it can only be assumed that the ordinary sources of infection are removed. The explanation of this is partly to be found in the exudations which run freshly only during May and June; and possibly when the life-history of the insects which infest the willow twigs is fully known it will help to clear up this matter.

THE PATHOGEN

It has been stated already that this disease is due to a bacterium inhabiting the watermarked tissues, but of a number of organisms to be found therein at various stages of the disease, one is always present when the first symptoms appear. It has been possible to prove this organism to be the pathogen, and I propose to call it, provisionally, *Bacterium salicis n. sp.* *Bacterium salicis* is a non-sporiferous, capsulate, aerobic and facultative anaerobic, short rod-shaped schizomycete making a rather viscid white filiform growth on beef-peptone-sucrose agar streaks, and on poured plates of the same medium, forming slowly, small circular, distinct, convex, smooth, shining, white, rather translucent and transparent surface colonies, with a plain edge. Under the microscope the larger colonies are resinous in colour and show a decided grumose internal marking which is sometimes interrupted by reticulations. A surface colony which has arisen from a buried one very typically shows internal lines which give it the appearance of having flowed out of the latter. The smaller surface colonies are transparent, vitreous, or oleaginous, and show fine granulations or are clear of internal markings. Buried colonies are circular, elliptical or triangular, oleaginous or resinous according to size, and on thickly sown plates may show a tendency to group together. Sometimes arborescent outgrowths are sent out from the side of buried colonies. On poured plates of beef-peptone-sucrose gelatine no liquefaction takes place. The colonies as on agar are slow growing and are circular, but are more deeply resinous and have a regularly and deeply fimbriate edge. They have a decided tendency to send short arborescent growths down into the gelatine. The agar stab shows a plain filiform growth down the whole length through which the needle penetrates. The gelatine stab is very typical for this bacterium, it begins as a plain filiform growth, but soon develops arborescent growths in

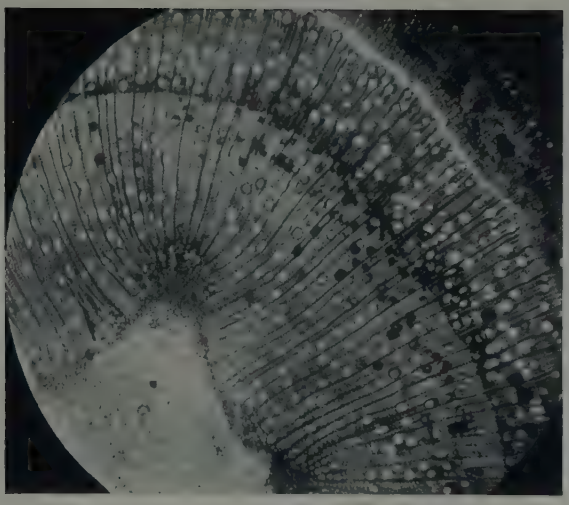

FIG. 11

From a two-year-old twig. The bacteria chiefly occupy the vessels near the pith. They have fallen out of a few vessels only.

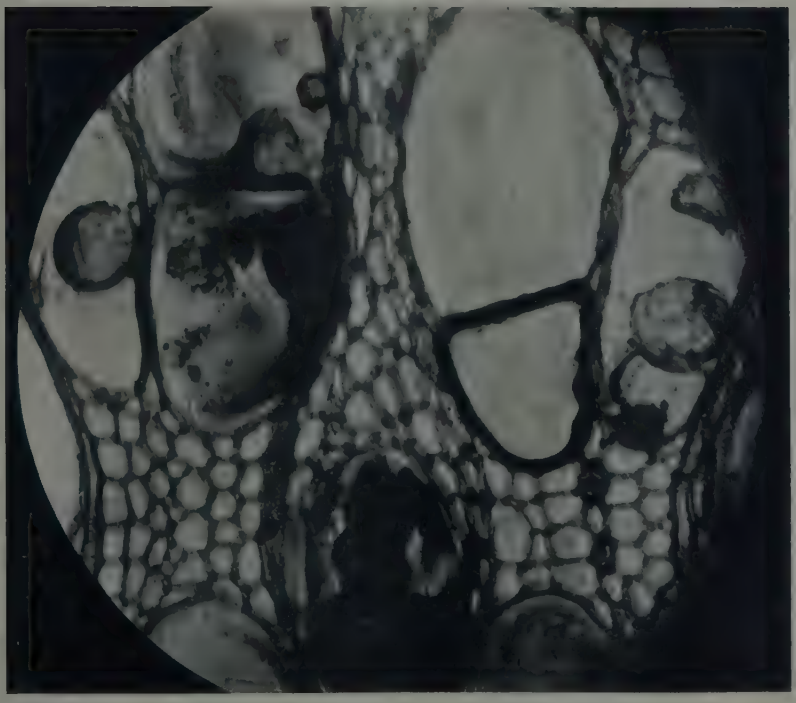

FIG. 12

Tyloses in root wood.

tufts down the side of the stab. In beef-peptone-sucrose broth a moderate turbidity is formed from which there is little or no precipitate: the culture does not clear itself, and forms no pellicle or froth. Steamed potato slants (in Buchner tube over water) are not immediately discoloured, and a typical bright yellow streak is developed which is spreading and runs round the base of the potato. The starch is attacked and after a time the streak turns darker in colour, becoming brown, and the potato is eventually discoloured brown. Milk is not coagulated, no acid or gas is produced on litmus milk. Nitrates are not reduced. No growth is obtained on Cohn's solution and little or no growth in Uschinsky's solution. On carbohydrate peptone media the following results were obtained. With dextrose sucrose, mannitol, and salicin there is a very strong acid reaction with a moderately turbid growth, but no pellicle. No gas, or only a very slight amount, is produced in the anaerobic arm of a fermentation tube or in a Durham's tube. After a time the turbidity clears, leaving a considerable red precipitate on the sides and bottom of the test-tube. With maltose there is a slight but decided production of acid with but little or no precipitate: the same holds good for glycerine except that the production of acid is even less. With lactose, no acid or gas or turbidity is produced: this corresponds with the growth in milk. Finally on no media is there any fluorescence or irridescence.

The agar, gelatine, and beef broth media were all standardized by the use of indicator solutions to a hydrogen-ion concentration corresponding to pH 7·2. The broth for this medium was made according to the following formula: beef extract 5 grm.; sucrose 1 grm.; peptone 10 grm.; sodium chloride 5 grm., dissolved in tap water and made up to 1,000 c.c. For making solid media 1·5 per cent. agar-agar or 10 per cent. gelatine was added. Sucrose was found to favour the growth of the pathogen, which will make quite a considerable amount of growth on sucrose solution in tap water or in soil extract water only. For all other media the standard formulae have been used.[1]

The limits of dimension of *B. salicis* are approximately $0{\cdot}3-0{\cdot}4\,\mu \times 0{\cdot}8-1{\cdot}0\,\mu$ if measured after being stained in the ordinary way. Measured in an Indian ink preparation it is usually twice as long and wide as this, owing to the capsule. This organism stains well with 1/10 saturated aqueous-alcoholic fuchsin and gentian violet, and with Ziehl's Carbol Fuchsin, but is rather feebly stained with 1/10 saturated aqueous-alcoholic methylene blue or Loeffler's methylene blue. It is Gram-positive and is not acid fast. It occurs singly or in chains of two, and I believe it to be motile, but have been unable to stain any flagella.

INOCULATION EXPERIMENTS

(*a*) TECHNIQUE OF ISOLATION

The pathogen is most easily isolated from branches in which the water-mark is still the colour of sap-logged wood. In these it is always predominant

[1] As in Eyre, *Bacteriological Technique* (W. B. Saunders & Co., 1916); or Giltner, *Microbiology* (John Wiley & Sons, 1921).

and very often occurs in pure culture. It appears to die after the watermark has changed colour, and many other organisms can then be isolated. These are much faster growing than the pathogen, which is very difficult to isolate from them.

In collecting material for purposes of isolation every precaution should be taken to procure clean unwounded branches, with no dead snags. Pieces of some length should be cut, and the isolation made from the middle of the piece or as far from a cut surface as possible. Branches 3/8″ in diameter or thereabouts are the most convenient to handle, but of course, any piece of suitably watermarked wood will do.

Before proceeding to make the isolation it is necessary to sterilize the exterior of the branch. This is most easily done by carefully passing it through the flame of a Bunsen burner, If the material is small and soft it is often safer to sterilize by washing with 1/1,000 solution of mercuric chloride. The isolation can be made most readily by taking advantage of the exudation of bacterial slime that comes naturally from watermarked tissue in its earlier stages, even after material has been cut from the tree and carried to the laboratory. All that is necessary is to cut the branch through in a fresh place, to take a loopful of the exudation, or of sterile water mixed on the cut surface with it, and from this to make a plate culture without delay. This method is quick and has the advantage in that no chance is given to saprophytes to suppress the pathogen.

When no exudation occurs the pathogen must be grown from a piece of the diseased wood. The great disadvantage of this is that any fast-growing species present tend to swamp the pathogen and prevent its isolation. Solid media may be used, but quicker results are obtained with liquid. Peptone-sucrose or beef-peptone-sucrose media are suitable : even pure sucrose solution is usually satisfactory. The sucrose favours the growth of the pathogen, and while it also favours the organisms that follow in the occupation of watermarked tissue, their growth on media containing it is sufficiently characteristic to enable them to be easily distinguished as a rule. Nearly all these organisms (*a*) produce gas from sucrose, (*b*) form a distinct pellicle, or (*c*) produce a dense turbidity; (*b*) and (*c*) are especially shown on beef-peptone-sucrose broth, in which medium the pathogen produces only a moderate turbidity with no gas or pellicle.

I made a number of attempts to isolate the pathogen from leaves taken off affected branches, choosing those that had just begun to wither. All these attempts failed, thus confirming what has been already observed, namely that the bacteria do not enter the vascular system of the leaves.

(*b*) DESCRIPTION OF EXPERIMENTS

A pure culture of the pathogen was used for all inoculation experiments, which are in number and type as follows:

(*a*) ***Direct inoculations into the vascular system.***

I. Five series of inoculations, made on cuttings.

II. Inoculation made on a pollard willow.

(*b*) *Attempted inoculation through parenchyma wounds, hydathodes, and stomata.*

I. Attempted infection of eight cuttings through the stomata, the parenchyma of the leaves, &c.

II. Inoculation in wounds in the bark only.

The material used for I (*a*), with the exception of twelve cuttings, and that used for II (*b*), was obtained by permission of Dr. Troup, Professor of Forestry, Oxford, from trees growing in Bagley Wood near Oxford. These trees were chosen because they are removed from any known source of infection and also because they had been specially planted as *Salix caerulea*. However, in 1923 I discovered that they bore male flowers and were only an exceedingly similar but distinct tree which is a variety of *S. alba*. Fortunately it was possible to check and confirm on true *S. caerulea* the results obtained on these cuttings. This was done in I (*a*) on the above-mentioned twelve cuttings. The material for these latter and for I (*b*) was obtained from a plantation of *S. caerulea* so far unaffected by the disease. The pollard willow used for II (*a*) is *S. caerulea* also, and was given for experiment by Dr. A. H. Church of Oxford.

I. (*a*) *Inoculations on 48 cuttings.*

The following table gives a summary of these inoculations. Infection was made by pricking directly through to the wood with an infected needle, in the same year in which the cuttings were struck, but only after strong root-and-shoot development was assured.

TABLE II

SUMMARY OF INOCULATIONS MADE ON CUTTINGS DURING 1922 AND 1923

Inoculation Reference.	*Time of Planting.*	*Species of Tree.*	*Date of Inoculation.*	*Culture used (Date of isolation).*	*Number of Cuttings Inoculated.*	*Number of successful Inoculations.*	*Number of Controls.*	*Number of Controls contaminated.*
1st Series	Jan. 1922	*Salix alba*	25. 3. 22	Feb. 1922	6	6	6	0
2nd Series	„ „	„ „	18. 8. 22	Aug. „	7	7	7	0
3rd Series	Jan. 1923	„ „	21,29. 3. 23	„ „	21	14	–	–
4th Series	„ „	„ „	8. 5. 23	„ „	6	5	6	1
5th Series	May 1923	*Salix caerulea*	16. 7. 23	May 1923	8	8	4	0
				Total	48	40	23	1

With slight variations, depending on the time of year in which the inoculation was made and the conditions under which the cuttings were kept, these experiments all gave similar results, confirming one another. Accordingly it is possible to give a general history which is true for each series of inoculations. This is done in the account below, in which it is assumed that inoculation took place in the spring. When cuttings are inoculated at a later date, the same series of events occurs with variations as to the extent to which some one or other of the symptoms of disease may develop. For instance, if cuttings are infected in August, adventitious shoots that develop will fail to grow to any extent, owing to the lateness of the season, when this symptom of disease appears.

All the cuttings were stuck in pots, in a cool green-house, and Series 1 and 2, 1222, were kept in there during the whole of the experiment. In 1923, owing to lack of room with the greater number of cuttings being used, all plants were transferred out of doors into the garden on May 7, or one week after inoculation.

Summary history of inoculations.

(1) For from six to ten weeks no symptom of disease appears. The first sign is then seen in the withering and death of the weaker shoots, and concurrently or slightly later of the lower leaves on the larger ones. Exceptionally, when no predominantly strong shoot has developed, all may die back together. Fig. 14 is a photograph of a typical cutting at this stage.

(2) At the same time as (1), or shortly afterward, adventitious shoots develop from the lower part of the cutting. These shoots grow much faster and often develop to be much greater than the original ones.

(3) After (2) and while the bark is still green, and in the majority of cases showing no sign of death or fungal attack, a cork layer begins to form, encircling the stem above the adventitious shoots.

(4) The plant now dies back to the cork layer. Exceptionally, more than one cork layer is formed, to which the cutting dies back successively.

(5) The dead parts of cuttings become attacked by fungi. In these, *Cytospora* predominates, as shown by the fructifications with which the dead parts are studded. A quite considerable space of time may elapse between the death of the tissues and the appearance of *Cytospora* pycnidia. This is a distinguishing feature from a plant dying back due to attack by this fungus, for then the formation of pycnidia always follows closely after the death of the bark.

(6) The wood is found at all stages to be only very slightly or not at all watermarked and no exudation of bacteria occurs from it. The development of bacterial masses is very slight, and only a very few, with considerable difficulty, can be found. On some but not all cuttings there is a watermark discoloration in the larger of the dying shoots.

(7) Bacteria can be isolated from the wood of all cuttings dying back: but in no case have I re-isolated *B. salicis*. It is to be noted, however, that among the different organisms obtained, I was able to identify some as being the same as those found to be followers on after the parasite in naturally infected tissues.

Allowing for the difference in size between these cuttings and a tree, the same symptoms are shown as a result of inoculation, as are seen in naturally infected trees. The cuttings die back, commencing with the withering of leaves and small shoots; adventitious shoots are developed, and a cork layer is then formed to which the whole plant dies back. But the most important things are lacking; the watermark is not properly developed, no exudation of bacteria occurs, and it is found impossible to re-isolate the inoculating organism. It became necessary, therefore, in order to definitely establish the pathogenity of *B. salicis* to reproduce these features of the disease. The great difference in size and condition between a small cutting in its first year and the large trees naturally infected, suggested the necessity of carrying out an

FIG. 13
Vessels completely blocked with tyloses.

FIG. 14

Fig. 14. A cutting inoculated with *B. salicis*. Photographed in June 1922, twelve weeks after inoculation. The smaller shoots have died and the leaves on the larger ones are also dying off.

inoculation on a well-established tree of some size; and for this reason the experiment now to be described was carried out.

II. *Inoculation of the pollard willow.*

This tree has a main stem seven feet high and seven inches in diameter. It had been pollarded in the late spring of 1923, and at the time of inoculation, July 25 of the same year, possessed a dense head of strong shoots, nearly fully grown. I chose for inoculation eight shoots, with secondary branches already developed on them, and having an average length of three feet six inches. The shoots were inoculated in the stem from beef broth cultures of *B. salicis*, using a hyperdermic syringe. Half were inoculated from an isolation made in August 1922, and the remainder from one made in May 1923. The tree is not shaded and is in a position that is well drained and free from damp and mists.

None of the inoculated shoots appeared to be affected until the end of September, when the leaves began to wither on one shoot in the centre of the crown, that had been infected with the May 1923 isolation. These leaves died, turned brown, and hung on the branch in exactly the same manner as do those on naturally infected trees. On October 9, when there was still no sign of death in the superficial tissues except that the soft tips of the shoot had withered, I removed the branch and found it to be very badly watermarked. The watermark was still in its earlier stage when it presents the appearance of 'sap-logged' wood, and a considerable exudation of sap containing bacteria occurred both from the cut branch and the stump remaining on the tree. This branch had been inoculated in the main stem at one place only, about eight inches from the tip, and the wound made by the syringe had healed over. From the point of inoculation the watermark had spread upwards two to three inches only, and downwards throughout the whole length of the branch into the main stem of the tree. No watermark was to be found in the side shoots or in the leaves, and there was no trace of any fungal infection whatever. On the same day that I removed the branch I made plate cultures from the bacterial exudation that occurred in cutting it through, even after it had been carried to the laboratory, and succeeded in isolating *B. salicis* in pure culture from the lower end of the branch, at about two feet away from the point of inoculation, and at intermediate places. I also dropped pieces of watermarked wood into nutrient broth and from the growth obtained therein made plate cultures in which the pathogen again appeared in pure culture.

Of the remaining branches I removed one growing in a similar position to the above, but inoculated with the August 1922 isolation and found that the inoculation had completely failed and the wound dried up and healed over.

III *and* IV. *Attempted inoculation through stomata, hydathodes, and wounds in the parenchyma of the leaf and bark.*

With regard to these it is only necessary to say that up to the present it has been impossible to reproduce any symptom of disease by any other means than direct inoculation into the vascular tissues. *B. salicis* appears to be incapable of attacking and disintegrating parenchyma.

General remarks on inoculations. Although the chief symptoms of the disease, as developed in the field, have only been reproduced on one plant and there only in one branch of the tree inoculated, they were in this case so

plain and definite and the re-isolation of the inoculating organism so successful that there can be no doubt that the pathogen has been discovered. The partially successful series of inoculations carried out on cuttings only go to confirm and to emphasize this. Two things are noteworthy with regard to the pollard willow: the branch which died back was in the centre of the crown; and it had been inoculated with the more recent isolation of the pathogen. The latter point suggests, especially in conjunction with the third series of inoculations on cuttings (Table II), that the isolation of the pathogen made in August 1922 was losing its virulence, through continued growth on artificial media. The former point emphasizes what has already been noted from observation in the field, viz. that the first branch to show symptoms of disease is quite frequently one heavily shaded by other branches, and supports the suggestion that such are particularly liable to become infected.

Incubation period. On the pollard willow, an incubation period of eight weeks was required, and in the various experiments on cuttings one from six to twelve weeks. I consider it to be very doubtful whether in the late spring under natural conditions so long a period is required as even six weeks, but until the history of the disease before the withering of the leaves begins is better known, no definite statement can be made regarding this. The conditions within the wood, when growth recommences, are very favourable to the rapid spread of the pathogen, owing to the quantity of sap and sugar present, and this seems to make an extremely rapid spreading of the disease possible at this time.

RELATION OF *CYTOSPORA CHRYSOSPERMA* (PERS.), FR. TO THE DISEASE

The close association of *Cytospora chrysosperma* with the dying branches of *Salix caerulea* affected by this disease, makes some discussion necessary as to the extent to which this fungus is a parasite and the work really done by it towards the death of the tree. The fungus belongs to a genus some species of which are known to cause the crown of trees to die back. It is associated in this respect with various species of the genera *Populus* and *Salix*, and has recently been reported in this connexion from the U.S.A. and Canada,[1] as causing die-back of the crown and canker in the stem. In these cases it seems to have acted as a low vigour parasite often following fire or other injury, when the trees must obviously have been in a decidedly weak condition. The evidence already put forward will have made it sufficiently plain that in this disease of *Salix caerulea*, *Cytospora* can only be regarded as a follower on after the true pathogen; and indeed that its presence is in no

[1] Long, W. H., 'An undescribed Canker of Poplars and Willows caused by *Cytospora chrysosperma*. *Journ. Agr. Research*, xiii. 331–43, May 1918.

Hubert, E. Ernest, 'Observations on Poplar Canker in the North-West'. *Phytopathology*, vol. x, pp. 442–7, 1920.

Poovah, H. H. W., 'Attack of Poplar Canker after Fire'. *Loc. cit.*, vol. xi, pp. 157–65, 1921.

Moss, E. H., 'Observation on two Poplar Cankers in Ontario'. *Loc. cit.*, vol. xii, p. 425, 1922.

way necesssary to bring about the dying back of the tree. There is a great contrast between the parasitic qualities of the pathogen and this fungus. Observation in the field shows that the pathogen is capable of attacking any tree; the more vigorous the tree, the worse the result of its attack. On the other hand, unless a tree is subjected to definitely unhealthy conditions, the fungus never causes it to die back. I give below a number of examples, besides that afforded by this disease, which illustrate this.

(1) In 1921, many willows at Langford in Essex were badly affected by the drought. They partly died back and *Cytospora* fructifications appeared over the whole of the dead branches, exclusive of other fungi. Since 1921 there has been a sufficient supply of water and no further dying back has taken place.

(2) A similar example was afforded by sets planted early in 1923. Many of these showed signs of distress during the hot, dry spring and early summer of that year. They were then watered and most of them entirely recovered, but a few that were more seriously injured died back apparently due to the attack of *Cytospora*—the pycnidia of which appeared in the bark. It was noteworthy that some of these sets recovered although the fungus had made considerable headway down the branches.

(3) The crowns of felled trees become infected and their death is hastened by this fungus.

(4) At the end of May 1923 I attempted to strike seventy-two cuttings of *Salix caerulea*. In order to expedite their growth I placed them in a cool but damp greenhouse. Nearly all of the plants started well but afterwards fifty per cent. died back due to the attack of *Cytospora*. When planted earlier in the year, and in the open, few or no cuttings die if carefully selected before planting. The high percentage of deaths in the greenhouse must be put down to the lateness of the season and to the favourable conditions for the growth of the fungus. Also experience seems to indicate quite definitely that pot-grown cuttings always make less vigorous growth than those planted out of doors in the nursery beds, even if otherwise the conditions of growth are the same. Similarly to the above some cuttings standing in water on the laboratory table were killed by *Cystopora*. Fig. 15 shows the stem of one of these.

Experiments with Cytospora chrysosperma.

At the beginning of this investigation I thought the disease to be due to this fungus, and began experiments from this point of view. The results of these so far as carried out are relevant to this discussion, and I give them below.

Experiments 1 and 2. The growth of the fungus on sterile dead material.

Short pieces of healthy branches enclosed in a test-tube and autoclaved were used for these experiments. Eighteen inoculations were made, and six controls were kept. The fungus grew well and formed numerous pycnidia, from all of which the typical orange-coloured spore threads were extruded.

Experiment 3. The growth of the fungus on pieces of live willow branch.

Fifteen inoculations were made and six controls kept. The pieces were entirely coated with paraffin wax after inoculation. All those infected died

in about six weeks. They began to die back round the point of infection, and *Cytospora* pycnidia appeared within a short time after the death of the bark. Eventually a great profusion of spore threads was produced from the pycnidia, and on three pieces small groups of the perithecial fructification appeared. *Valsa sordida*, formed a long time after the appearance of the pycnidia. The control pieces showed no sign of dying back, but developed roots and shoots as would normal cuttings. On those inoculated no such development occurred.

Experiment 4. The attempted infection of six cuttings planted in pots. Six controls were kept.

Date of inoculation, 25 March 1922.

The whole experiment was carried through in a cold house. One cutting died completely thirteen weeks after inoculation. One other first of all died back slightly round the point of inoculation, and then slowly died down to the soil level. At the same time a strong new shoot was put up which remained entirely unaffected by the fungus. The four remaining cuttings were quite unaffected, as were also the controls.

In all the above experiments inoculation was made by inserting beneath the bark a piece of mycelium from a pure culture of the fungus grown on malt extract agar-agar. In none of the experiments did any sign of watermark appear.

From the experimental work, and from the various other observations recorded, the following conclusions may be drawn:

(1) That *Cytospora chrysosperma* is a saprophyte and capable of normal development on dead tissues.

(2) That it is a facultative parasite and can attack and kill live tissues providing they are in a weak condition and predisposed to disease.

(3) That it is probably quite incapable of successfully attacking any vigorously healthy plant.

(4) That should a tree become diseased it is liable to be attacked by *Cytospora*, which probably causes death somewhat sooner than would otherwise have been the case. This point sums up the relation of the fungus to the watermark disease: even on a badly watermarked tree, it has never been observed to attack any branch not already dying back.

(5) That *Cytospora* cannot be regarded as a dangerous parasite on *Salix caerulea*, and that it has a purely secondary action following on after more powerful causes of disease.

MEANS OF PREVENTION

The habitat of Salix caerulea. Before discussing means of prevention it is necessary that some description should be given of the habitat of the host. The remarks made here apply to the lower Chelmer valley, between Chelmsford and the sea, and in particular to the districts around Little Baddow and

Langford. The disease is also prevalent in parts of Hertfordshire, and while the same general statements will hold good for this county, the smaller details are liable to be different.

For the cultivation of *Salix caerulea*, a soil is demanded which is fertile, provided with abundant moisture and good drainage. Rich alluvial land by the side of running water is considered to be best, but any good loamy soil that is well watered and drained is very suitable for its cultivation. Judging from the position in which it is usually planted, the general opinion is that *Salix caerulea* requires a locality in which the water level is near to the surface. I believe this to be a mistake, and that the optimum locality for healthy and rapid growth is one where the soil is fertile and moist throughout the year, but where the water level is definitely below the depth chiefly occupied in root development. On such a soil the roots are kept permanently and well aerated and the tree has the maximum opportunity for healthy growth and development.

In the Chelmer Valley, the tree is to be found along the banks of the river or its tributaries (Fig. 16), and as a hedgerow tree, by the side of well-watered meadows (Fig. 17) or of the arable fields lying on the lower slopes of the valley (Fig. 1). In the latter case the trees are usually planted by the side of permanently running ditches. The sub-soil of the river banks and meadows is alluvium, except on one bank of the Chelmer, where much gravel has been thrown to form a towing-path. That of the arable fields is glacial deposit of gravel, sand or loam, or of river gravel, both overlying London clay ; or sometimes of London clay itself, which is then usually much alleviated by the down-wash from the lighter soils higher up. These lower fields receive the seepage from the higher land ; and because of this usually possess a subsoil which is always well watered, even in very dry seasons. Under these circumstances *Salix caerulea* is rarely or never affected by drought, but is much more liable to suffer from an excess of wate.

The propagation [1] of the tree is carried out by means of large sets cut from pollard willows or the tops of healthy trees on felling. Owing to the considerable economic value of the timber, there is a tendency to plant wherever an available space is to be found, and for this reason many plantations occur on land unfit for agriculture, usually because too wet. The most suitable formation for planting is the single row, because it gives the maximum of light and air and space for crown development necessary for the healthy cultivation of *Salix caerulea* and for the production of the required type of timber. The planting distance between adjacent trees varies according to the district, but is usually either ten or fifteen feet: that is, it is the minimum at which it is estimated the required type of timber can be grown.

Proposals for the prevention of the disease. Before proceeding to enumerate the measures necessary for the prevention of the disease, some proposals arising out of the method of cultivation of the tree and the locality on which it is planted must be discussed.

The disease in relation to locality. In the Chelmer valley, the disease appears to have become epidemic first among the river-side plantations and to

[1] For a description of *Salix caerulea* and a summary of the method of its cultivation see Elwes & Henry, *Trees of Great Britain and Ireland*, vol. vii.

have spread from these up the sides of the valley. The chief devastations have occurred in the damp riverside meadows; most trees still remaining undiseased in the more badly affected districts being on the better drained and drier ground. I think it is true to say, generally, that wherever a great loss has taken place there is a definitely wet subsoil, abundantly supplied with water throughout the year. On the contrary, it is the localities with a moist, but never sodden, subsoil that have been freer from attack. The disease appears, therefore, partly to resolve itself into a water problem: those localities with a superabundant supply of water in the subsoil being most likely to induce it. Further investigation is required as to the exact relationship between the disease and the water-content of the soil and subsoil; but the general evidence provided is sufficient to make it necessary that, for the time being, localities which are definitely wet and inclined to be marshy, or where the water level throughout the year is but a short distance from the surface, should not be planted upon. Fig. 16 shows what is probably an ideal situation for willow growing. Here the trees are by the side of a small stream and on a bank some three feet above the normal level of the water. The soil is good, well drained and provided with abundant moisture, but it is to be noted the adjacent fields are not wet or marshy. No disease has appeared in this plantation.

Apart from the above, the disease is not to be associated with any particular locality; but no sets should be planted where the soil is known to be unsuitable. Generally speaking, wet marshy localities, particularly if the water is stagnant, peaty soils, clays or other impermeable soils, and usually gravels, should be avoided.

The disease and the planting distance. Sets are planted at present so close together that the crowns of the trees meet by the time they are five to seven years old. And at the end of the rotation, their shape when grown in single rows is elliptical, instead of circular as in the case of an isolated tree. In any one lot of trees planted at the same time some are slower growing than others, and certain trees become dominant and tend to suppress their neighbours. If a single row of such trees is kept under observation it is found in the majority of cases that the first symptoms of disease appear either in the crowns of the partially suppressed trees or in a heavily shaded branch of one of the dominant crowns. There are exceptions to this rule, but it applies to a sufficient extent to show that some association exists between overshadowed and suppressed branches, and liability to infection. Accordingly I consider it advisable in localities that have been subject to disease, if nowhere else, that sets should be planted further apart than is at present the practice. Thirty feet is, I believe, the maximum planting distance yet advocated.[1] This would allow free crown development for each tree; and when plantations of *Salix caerulea* are made, I consider that trees should be this distance apart. If single rows only are planted, it is possible that a smaller distance between tree and tree could be adopted. In no case should this be less than twenty feet, but twenty-five feet is preferable, especially where the disease is prevalent. The efficacy of planting at a wider distance can admittedly only be tested by experience. It should,

[1] Elwes & Henry, *Trees of Great Britain and Ireland*, vol. vii, and Pratt, E. R., *Journ. Roy. Agric. Soc.*, lxvi, pp. 19–34 (1905).

however, be remembered that *Salix caerulea* is a very strongly light-demanding tree, which is easily injured by shade, and, contrary to many forest trees, is unsuitable for growing in close canopy. Anything which causes the crown to be overshadowed injures and weakens the branches shaded; and, as is common experience, it is such parts that are especially open to attack by parasites, both plant and insect.

General statement of preventative measures. Apart from the above somewhat controversial measures, which have yet to be tested by experience, others without doubt are necessary. These are included in the summary statement given below of all measures proposed.

(1) Trees at present diseased to be felled, the tops burned, and the remaining parts removed from the vicinity of plantations.

(2) Old and rotten pollards and any other trees that may be suspected to provide suitable hosts for the pathogen, to be entirely cleaned out of plantations and all other places.

(3) Each season trees freshly showing symptoms of disease to be felled, the tops burned, and other parts removed if possible. If it is considered impossible to fell in the late Spring or early Summer, then these trees must be destroyed in the Autumn and by no means left over until next Spring to become centres of infection. I consider felling at the time the disease appears to be important, as it prevents any immediate spreading of infection.

(4) Only carefully selected, healthy, vigorous sets should be planted.

(5) No unsuitable soil to be planted upon. In this it is to be remembered that *Salix caerulea* is a surface rooting tree, and that even a thin top layer of the wrong soil may be sufficient to make a locality unsuitable. Careful attention to soil requirements is important, particularly where there is a wet subsoil.

(6) No soil, the subsoil of which is wet and sodden, showing that a considerable excess of moisture is present, to be planted upon.

(7) That the planting distance between tree and tree be increased to thirty feet in the case of plantations and to twenty to twenty-five feet in the case of single rows of trees.

It is advisable that these proposals should be carried out over the whole of affected areas; and this applies especially to the removal of diseased trees. Any grower who leaves infected trees standing is endangering not only the trees on his own land, but those of all others in the district.

DISCUSSION

Comparison with similar diseases. No other such disease of trees appears to be recorded, with the result that the vascular bacterial diseases of herbaceous plants are the only ones with which comparison can be made. This disease is essentially similar in type to these, but differs principally (*a*) in the rapidity of the dying back, (*b*) in the proportion of vessels blocked up by the pathogen, (*c*) in the extent to which the parenchyma is attacked.

(*a*) As a rule an herbaceous plant wholly dies back during one season.[1] In this disease, only a limited dying back occurs because although the one-year-old wood of the still living parts of the tree may become diseased, that of the current year is left open to carry sap. In a herbaceous plant there is no opportunity for such restricted spreading of disease, and the whole vascular system becomes affected.[2]

(*b*) Herbaceous plants often have the majority, if not the whole, of the vessels in any one section of stem blocked up with bacteria. In this disease only a minority are so affected, and presumably this is because a smaller proportion are required to carry sap [3] and smaller food supplies are available in comparison with the size of the plants. In a herbaceous plant there is a greater capacity for food storage than in a tree in proportion to the number of vessels, and accordingly a greater amount of food reserve upon which the bacteria can draw. That they do this is indicated [4] in this disease by the absence of starch from the wood of watermarked branches, at least in the vicinity of the watermark, and by the greater extent to which in the twigs the vessels are occupied which are adjacent to the large stores of starch normally present in the outer cells of the pith.

(*c*) This disease is exceptional [5] in that the parenchyma appears never to be disintegrated. The woody nature of the plant is partly the cause of this, but the pathogen appears to be incapable of attacking parenchyma even when directly inoculated into it.

Rate of spread of disease in relation to the wood sap. In this disease there appears to be correlation between the rate of spread of the pathogen and the quantity and quality of the wood sap, which is most abundant and is richest in organic substances about the time of the bursting of the buds.[6] The pathogen spreads through the tree chiefly before the first symptoms of disease appear, and during the remainder of the season makes but slow progress. It is very noteworthy that this slow rate of spread coincides with the existence of negative pressure within the vessels and the diminution from the Spring maximum,[6] in the quantity of sap and of sugar in the sap that accompanies this. An explanation seems here to be offered for the failure of the pathogen to extend sufficiently to cause further branches to die back after July. But it cannot be held to explain why fresh trees do not die back, as the successful inoculation of the pollard willow has proved that this may occur, at least up to the end of September.

[1] As for example in the 'Cucurbit Wilt', described in *Bacteria in Relation to Plant Diseases*, Erwin F. Smith, vol. ii, p. 208; Stewart's 'Disease of Sweet Corn (Maize)', *loc. cit.*, vol. iii, p. 88; 'Brown Rot of *Solonaceae*', *loc. cit.*, vol. iii, p. 174.

[2] Examples of this are given by E. F. Smith, *loc. cit.*, vol. ii, p. 250, for the 'Cucurbit Wilt', and vol. iii, p. 177, for the 'Brown Rot of *Solonaceae*, on a potato plant'.

[3] See Dixon, *Transpiration and Ascent of Sap*, pp. 93 and 94.

[4] For similar instance of this see Smith, *loc. cit.*, vol. iii, 199, fig. 108, showing absence of starch in vicinity bacterial foci.

[5] E. F. Smith, vol. ii, p. 69, states that all vascular diseases make pockets in the parenchyma. No such pockets appear in this disease. The bacteria only escape from the vessels within the protoxylem and these are normally ruptured in the growth of the twig, thus providing means of escape.

[6] See W. R. G. Atkins, *loc. cit.*, p. 192.

The effect of by-products of the pathogen. The primary cause of this disease is perhaps to be found in the mechanical blocking of the vessels. There are, however, two features which are most easily explained as being due to the toxic effects of the by-products of the pathogens. They are (*a*) the abnormal formation of tyloses and (*b*) the premature leaf-fall.

(*a*) The abnormal formation of tyloses is common to vascular bacterial diseases. The view that it is due to the by-products of the pathogen has been put forward by E. F. Smith,[1] who regards overgrowths of parenchyma in general to be due essentially to oxygen hunger and an abnormal acid condition of the cell sap.[2] In this disease two results of the metabolism of the pathogen appear to confirm this: they are (1) the production of acid from the sugars in the wood sap, and (2) the setting up of anaerobic conditions within the wood.

(1) The pathogen, even in very weak solutions of sucrose, dextrose, and laevulose, produces a very strongly acid reaction to litmus. In the same way, in the wood sap, acid is produced from these sugars and thus its normal reaction, which is neutral to litmus, is changed.

(2) The anaerobic condition of the atmosphere within diseased trees is indicated by the very slow rate at which the colour change in the watermark takes place, and the much greater speed with which the same phenomenon is completed where watermarked wood or the exudation from it is exposed to the air. Apart from this, the pathogen is an aerobe, although also a facultative anaerobe, and will accordingly make great demands on the limited oxygen supply.

From the above it will be seen that whereas normally the wood sap is neutral to litmus[3] and the atmosphere within the wood aerobic,[4] in watermarked wood and to some extent in all adjacent tissues there is (*a*) a shortage of oxygen and (*b*) wood sap with an abnormally acid reaction.

(*b*) *Premature leaf-fall.* This is not due to water shortage, for none of the vessels of the current year's wood of branches affected by it is blocked up with bacteria. It is, however, usual to find an extensive formation of tyloses in this wood, and this indicates the existence of the abnormal conditions referred to above. Some confirmation is given to the view that this phenomenon is due to the existence of these conditions by the result of experiments carried out by Dixon.[5] He found that if a portion of the stem of a shrub was killed, the leaves above it gradually died, beginning with the lower ones, and therefore in the same manner as on branches affected by this phenomenon. Further, it was possible to show their death to be due to toxic substances released into the sap from the medullary ray and other parenchyma cells within the killed portion of stem. This suggests that the premature leaf-fall induced by this disease may not only be due to the by-products of the pathogen, but also to substances released from the parenchyma within the wood as a result of their action.

[1] E. F. Smith, *Bacterial Diseases of Plants*, p. 477.

[2] E. F. Smith, *loc. cit.*, p. 510 et seq.

[3] W. R. G. Atkins, *loc. cit.*, p. 190.

[4] Pfeffer, *Physiology of Plants*, Ewart's Transl., vol. i, p. 206; P. Sorauer, *Handbuch der Pflanzen-Krankheiten*, Vierte Auf., Band 1, pp. 92 and 93.

[5] Dixon, *Transpiration and Ascent of Sap*, p. 65.

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SUMMARY

(1) In this paper the result is given of an investigation into a disease epidemic within the last few years on *Salix caerulea*, the Cricket-Bat Willow, in the counties of Essex and Hertfordshire. This investigation has been carried out in Essex in the lower valley of the River Chelmer. The disease appears as a die-back of the crown and chiefly affects the larger trees, which it renders almost worthless.

(2) The first symptom of disease, which generally appears in May but occasionally up to the end of July, is the general wilting and withering of the leaves on a part, but never the whole, of the crown. The withered leaves turn brown and hang on for some time. The disease does not generally advance further during the first year, but takes two or more years to bring about the final death of the tree.

(3) Upon the death of the leaves, adventitious shoots frequently develop both upon the dying branches and other parts of the tree; but only those on the latter survive until next season. At this time also, thin sticky colourless exudations of bacteria, which turn brown on exposure to the air, occur on the dying branches. The exudations flow from the wood through wounds in the bark. The wounds are usually due to insects and never to the exudation bursting out of its own accord. It can easily be shown that the exudations have their origin in masses of bacteria occupying the vascular tissues. It is to be noted that at this time no fungus is associated with the dying branches.

(4) The bark of affected branches only dies back gradually. During the summer it becomes infected with a fungus, *Cytospora chrysosperma*, which hastens its death, and by the autumn or winter has thickly studded the dead bark with its fructifications.

(5) On cutting through an affected branch, the area occupied by the bacteria is seen to be darkly stained. The colour varies from that of 'sap-logged' wood, in the earlier stages of disease, to a dark brown or black in the later stages. When in the earlier stages, an exudation similar to those already mentioned flows from the stained regions, which because of this are known as the 'watermark'. The change in colour from white to brown observed in the exuded bacterial slime also takes place naturally within the wood, but much more slowly.

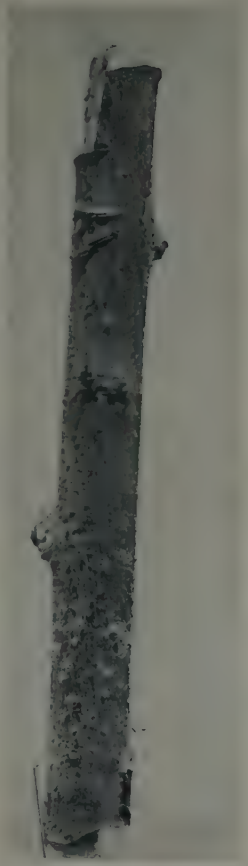

FIG. 15

Spore tendrils of *Cytospora chrysosperma* (*Pers.*) *Fr.* on the stem of a cutting.

FIG. 16

Salix caerulea growing by the side of a small stream. Photographed July 1922.

FIG. 17

A single row of trees, some of which are diseased, growing in the hedgerow of a low-lying meadow. Photographed July 1922.

(6) One particular organism can always be isolated, very often in pure culture, from freshly dying branches. This is a short rod-like, schizomycete, which is provisionally called *Bacterium salicis*. A description of this organism, in so far as it is at present investigated, is given in the paper. Inoculation experiments with *Bacterium salicis*, in pure culture, have demonstrated that it is the cause of the disease.

(7) This organism is a strictly vascular parasite which apparently enters through wounds in the smaller branches. It is confined to the larger vessels, and has so far not been found in the vascular system of the leaves or finer rootlets. Also it is apparently quite unable to attack and disintegrate the parenchyma or the woody tissues of the plant.

(8) Infection always takes place in the crown. The wounds through which the parasite enters are usually made by insects, which may themselves be carriers of disease. After the spread of the disease has begun, the exudations undoubtedly provide a source of infection from which the bacterium is spread from tree to tree.

(9) The dying back is chiefly due to stoppage of water-supply, and for this reason the dying of the leaves takes the form of a general wilt over the branches affected. The whole crown never wilts at one time for two reasons: (*a*) the bacteria are chiefly to be found in the last year's wood, thus leaving the current year's wood open for the supply of water; and (*b*) this is because the bacteria spread in a radial direction with extreme slowness, so that the disease does not extend from the one-year-old ring to that being laid down in the current season, and so cannot cut off the water-supply. A second, and usually secondary, cause of death is the poisoning of the sap (*a*) by the by-products of the bacteria, (*b*) by substances from the parenchymatous cells in the wood that have died due to the disease.

(10) The fungus, *Cytospora chrysosperma*, which is associated with the dying branches, is shown by observations in the field and experimental evidence, to be a saprophyte and facultative low vigour parasite, which is of itself unable to cause the death of a vigorously healthy tree.

(11) Observations in the field appear to show that trees growing in a locality with a definitely wet subsoil are more liable to disease than those on moist but well drained, or even dry subsoils. Also, the spread of the disease is favoured by the too close planting together of trees. This results in the partial suppression of the crowns, and the dense shading of some branches. It is usually in these suppressed parts that the disease first appears. Both these conclusions need to be confirmed by statistical observation.

(12) Various measures of prevention are suggested. These are chiefly measures of general hygiene, which are at present too often grossly neglected. Besides these, it is suggested that trees be planted further apart, in order to prevent mutual overcrowding, and that localities with a wet subsoil and which are inclined to be marshy be not planted upon.

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